Journal of the American College of Cardiology
© 2002 by the American College of Cardiology Foundation
Published by Elsevier Science Inc.

Vol. 40, No. 2, 2002
ISSN 0735-1097/02/$22.00
PII S0735-1097(02)01956-3

Endothelial Function Fluctuates With Diurnal Variation in the Frequency of Ischemic Episodes in Patients With Variant Angina

Hiroaki Kawano, MD, PHD,* Takeshi Motoyama, MD, PHD,* Hirofumi Yasue, MD, PHD,† Nobutaka Hirai, MD,* Hesham M. Waly, MD, PHD,§ Kiyotaka Kugiyama, MD, PHD,‡ Hisao Ogawa, MD, PhD*

Kumamoto and Kohfu, Japan; and Mansoura, Egypt

OBJECTIVES The aim of the present study was to investigate whether there is diurnal fluctuation in the endothelial function of patients with variant angina (VA).

BACKGROUND Coronary spasm is induced by acetylcholine and is promptly relieved by nitroglycerin. Thus, it is possible that endothelial dysfunction is involved in the pathogenesis of coronary spasm. Furthermore, the frequency of ischemic episodes is known to display diurnal variation.

METHODS Flow-mediated, endothelium-dependent vasodilation of the brachial arteries was measured in the early morning (6 AM), afternoon (2 PM) and evening (8 PM) in 20 patients with VA (mean age 54.5 years; 10 men and 10 women) and in 20 control subjects (mean age 54.2 years; 10 men and 10 women). All patients underwent 24-h ambulatory electrocardiographic monitoring during the study.

RESULTS Flow-mediated vasodilation in patients with VA was deteriorated by the early morning and improved by the afternoon (patients with VA at 8 PM vs. 6 AM vs. 2 PM: 7.8 ± 2.1% ($p < 0.01$ vs. VA at 6 AM) vs. 5.4 ± 2.3% vs. 8.8 ± 1.9% ($p < 0.01$ vs. VA at 6 AM); control subjects: 9.5 ± 2.8% vs. 9.0 ± 2.2% vs. 9.9 ± 1.9%, respectively). The frequency of spontaneous ischemic episodes was highest from midnight to morning and was lowest from morning to late afternoon (4 PM to midnight: 7 episodes; midnight to 8 AM: 25 episodes; 8 AM to 4 PM: 3 episodes).

CONCLUSIONS There is diurnal fluctuation in endothelial function, which is associated with variation in the frequency of ischemic episodes. (J Am Coll Cardiol 2002;40:266–70) © 2002 by the American College of Cardiology Foundation

Spasm of an epicardial coronary artery (i.e., coronary spasm) is well known to play an important role in the pathogenesis of not only variant angina (VA) but also other forms of angina pectoris and acute myocardial infarction (1,2). We, as well as other investigators, have reported that coronary spasm is induced by acetylcholine (ACh), which causes vasodilation when the endothelium is functioning normally, and the spasm is promptly relieved by nitroglycerin (NTG), which causes vasodilation through its direct action on the smooth muscle (3–6). This phenomenon suggests the possibility that patients with coronary spasm have a disturbance in the endothelial function of their coronary arteries (7).

The frequency of ischemic attacks displays diurnal variation in patients with VA (i.e., coronary spastic angina). The number of attacks increases in the early morning and decreases in the afternoon (7,8). If endothelial function is involved in the occurrence of coronary spasm, endothelial function may also fluctuate according to this diurnal variation. Thus, the present study was designed to examine whether there is diurnal fluctuation in the endothelial function of patients with VA.

METHODS

The present study included 20 patients with VA (mean age 54.5 years [range 33 to 63 years]; 10 men and 10 postmenopausal women) in whom recurrent attacks of chest symptoms occurred spontaneously at rest, with ST-segment elevation on the electrocardiogram (ECG), that was rapidly relieved by NTG. Coronary angiography was performed before the study, using the Judkin's technique. All patients had proven coronary spasm as demonstrated by chest symptoms and ST-segment elevation after intracoronary injection of ACh (3,7,9). The study also included 20 control subjects (mean age 54.2 years [range 32 to 62 years]; 10 men and 10 postmenopausal women). These subjects were selected to match the risk factors for atherosclerosis in the patients with VA. The control subjects underwent diagnostic cardiac catheterization for evaluation of chest symptoms. None showed coronary spasm after the intracoronary injection of ACh. None had any organic coronary stenosis, including irregularities of the lumen, by coronary angiography. The patients' characteristics are shown in Table 1. All medications, except sublingual NTG, were discontinued from at least seven days before study entry to completion of the study. All subjects gave written, informed consent, and

From the *Department of Cardiovascular Medicine, Kumamoto University School of Medicine, Kumamoto, Japan; †Kumamoto Aging Research Institute, Kumamoto, Japan; ‡Second Department of Internal Medicine, Yamanashi Medical School, Kohfu, Japan; and §Mansoura University Faculty of Medicine, Mansoura, Egypt. This study was supported in part by a Grant-in-Aid for science research (14770318) from the Ministry of Education, Science, Sports and Culture in Japan and a Grant from the Japan Cardiovascular Research Foundation, Tokyo, Japan.

Manuscript received October 4, 2001; revised manuscript received March 26, 2002, accepted April 17, 2002.

Abbreviations and Acronyms

ACh = acetylcholine
ANOVA = analysis of variance
ECG = electrocardiogram/electrocardiographic
NTG = nitroglycerin
VA = variant angina

the study was approved by the ethics committee of our institution.

Evaluation of myocardial ischemia. Myocardial ischemia of VA is often asymptomatic (7). To evaluate the frequency of both symptomatic and asymptomatic spontaneous ischemic episodes, all subjects had 24-h ambulatory ECG monitoring (Nihon Kohden, Tokyo, Japan) at 4 PM. The subjects were also instructed to record any symptoms during the study. An ischemic episode was defined as a period of ST-segment depression (horizontal or downsloping) of ≥0.1 mV or ST-segment elevation ≥0.2 mV, lasting ≥1 min before recovery to baseline, and was separated from another episode by an interval of ≥2 min.

Vascular studies. Increased blood flow during reactive hyperemia after transient occlusion causes vasodilation, mainly by releasing endothelium-derived nitric oxide or its related substances (10,11). Thus, endothelial function was evaluated by flow-mediated, endothelium-dependent vasodilation of the brachial arteries, using ultrasonography. The validity of this method has been confirmed in our previous studies and in other studies (12–15). The observation was performed in the evening (8 PM), in the following early morning (6 AM) and in the afternoon (2 PM), coincidentally with 24-h ambulatory ECG monitoring. Subjects were instructed to lie on a bed before and during the interval between the observations, except for mealtime or rest room necessity. Vasoactive substances, including beverages containing caffeine, were withdrawn. All observations were performed in a quiet, temperature-controlled room (22°C to 23°C) by two skillful examiners who had no knowledge of the subjects' grouping.

The brachial artery above the bifurcation, in the nondominant arm, was scanned in a longitudinal fashion, using a 7.5-MHz linear-array transducer (SONOS 2500, Philips, Amsterdam, The Netherlands). Optimal images were obtained between 1 and 5 cm above the antecubital crease. This location was marked, and all subsequent images were obtained at the same location. To ensure that measurements were taken at the same point of the brachial artery for the three observations, the exact distance from the antecubital crease to the mark was recorded for each patient. All machine operating parameters, including depth and gain settings, were optimized for each patient and were kept constant throughout the three observations.

The subjects laid quietly for 10 min before the first scan. After baseline measurements of the diameter and flow velocity of the brachial arteries, a blood pressure cuff placed around the forearm, below the bifurcation, was inflated to a pressure of 250 to 300 mm Hg. After 5 min, the cuff was released. Measurements of the diameter and flow velocity were continuously performed between the cuff inflations and after the cuff deflation. Thereafter, the subjects laid quietly for 15 min. After confirming that the diameter and flow velocity returned to baseline levels, sublingual NTG was administered (0.3 mg), and 3 to 4 min later, the last measurements were performed.

B-mode ultrasound images were recorded on a super-VHS videocassette recorder (BR-S601M, Victor, Tokyo, Japan). The diameter of the artery was measured at a fixed distance from an anatomic marker, using ultrasonic calipers. The measurements were taken from the anterior to posterior "m" line (the interface between the media and adventitia) at end diastole, coincident with the R-wave on a continuously recorded ECG (12,14,15). The diameter at four cardiac cycles was analyzed for each scan, and the measurements were averaged. The diameter measurements for reactive hyperemia were taken 45 to 90 s after the cuff deflation to measure the peak diameter (12,14,15). The response of the vessel diameter to reactive hyperemia and NTG was expressed as the percent increase in relation to the baseline diameter value. Volumetric blood flow was calculated by multiplying the velocity-time integral of the Doppler flow signal by the heart rate and vessel cross-sectional area. The percent increase in the blood flow of the brachial artery, observed immediately after the cuff deflation, was calculated as the maximal flow recorded within the first 15 s after the cuff deflation divided by the flow at baseline (12–15).

In our study, the interobserver variability for the repeated measurements of the arterial diameter at rest was 0.05 ± 0.02 mm. The intraobserver variability for the repeated measurements of the arterial diameter at rest was 0.02 ± 0.02 mm. In a preliminary study, when these procedures were performed at the same time on two separate days in 20 volunteers, the average intrasubject test-retest difference for the measurements of the arterial diameter during reactive hyperemia was 0.05 ± 0.04 mm (12,14).

Blood sampling and assays. Blood samples were obtained from the subjects while they were in the fasting state, before the ultrasound studies. Serum total cholesterol and triglyceride concentrations were measured enzymatically, and the

Table 1. Patient Characteristics

	Control Subjects (n = 20)	Patients With Variant Angina (n = 20)	p Value
Age (yrs)	54.2 ± 9.8	54.5 ± 8.8	NS
Men/women	10/10	10/10	
Smoker	9 (47.4%)	9 (47.4%)	NS
Hypertension	3 (15.8%)	3 (15.8%)	NS
Total cholesterol (mg/dl)	199.8 ± 14.4	197.9 ± 15.8	NS
HDL cholesterol (mg/dl)	52.0 ± 6.2	51.7 ± 5.8	NS
LDL cholesterol (mg/dl)	123.0 ± 10.2	122.7 ± 11.8	NS
Triglyceride (mg/dl)	110.0 ± 10.4	109.0 ± 9.8	NS

Data are expressed as the mean value ± SD.
HDL = high-density lipoprotein; LDL = low-density lipoprotein.

Table 2. Hemodynamic Parameters

	Control Subjects (n = 20)			Patients With Variant Angina (n = 20)		
	Evening (8 PM)	Early Morning (6 AM)	Afternoon (2 PM)	Evening (8 PM)	Early Morning (6 AM)	Afternoon (2 PM)
Heart rate (beats/min)	65.1 ± 10.9*	58.8 ± 10.0	66.1 ± 9.9*	64.7 ± 9.1†	57.7 ± 8.0	65.8 ± 10.0†
Mean blood pressure (mm Hg)	93.8 ± 6.7	92.8 ± 7.7	93.9 ± 5.0	90.1 ± 7.9	91.4 ± 9.4	91.1 ± 6.8
Baseline diameter (mm)	3.70 ± 0.19	3.67 ± 0.16	3.72 ± 0.15	3.69 ± 0.15	3.59 ± 0.18	3.68 ± 0.14
Baseline flow (ml/min)	222.4 ± 20.9‡	168.5 ± 16.8	232.4 ± 23.2‡	204.8 ± 24.1§	159.3 ± 15.6	210.8 ± 25.7§
Increase in flow during reactive hyperemia (%)	271.3 ± 20.4	289.4 ± 28.1	260.3 ± 26.9	255.3 ± 25.7	257.3 ± 25.4	240.3 ± 24.3
Increase in diameter after nitroglycerin (%)	17.0 ± 3.8	17.8 ± 5.6	16.8 ± 3.0	17.0 ± 4.7	18.1 ± 5.1	17.9 ± 3.7

*$p < 0.02$ vs. early morning (6 AM) in control subjects. †$p < 0.02$ vs. early morning (6 AM) in patients with variant angina. ‡$p < 0.01$ vs. early morning (6 AM) in control subjects. §$p < 0.01$ vs. early morning (6 AM) in patients with variant angina. Data are expressed as the mean value ± SD.

serum high-density lipoprotein cholesterol concentration was measured by heparin-Ca^{2+}/Ni^{2+} precipitation (14,16).

Statistical analysis. The characteristics of the patients were compared using the two-tailed, unpaired *t* test for continuous data and the chi-square test for group data. Analysis of variance (ANOVA) was used to compare the values of the myocardial ischemic episodes. When statistically significant effects were found, the Newman-Keuls test was used to isolate the differences between the groups. Two-way ANOVA with repeated measures, followed by post-hoc testing with the Scheffé's test, was used to compare the values of the ultrasound studies between the two groups. Correlation between flow-mediated vasodilation and myocardial ischemic episodes was made using linear regression analysis. Statistical significance was set at $p < 0.05$. Data are expressed as the mean value ±SD.

RESULTS

The baseline variables are shown in Table 2. The baseline values of heart rate ($p < 0.02$) and brachial blood flow ($p < 0.01$) were greater in the evening and afternoon than in the early morning in both the patients with VA and the control subjects. The baseline values of mean blood pressure were comparable among the three studies in both groups. Although the baseline diameter tended to be greater in the evening and afternoon than in the early morning, the differences were not significant in either group.

Every patient with VA had chest symptoms with ST-segment elevation during 24-h ambulatory ECG monitoring. Figure 1 shows the diurnal variation of the total and mean ischemic episodes in all 20 patients. The frequency of ischemic episodes was highest from midnight to morning (midnight to 8 AM: total 25 episodes, mean 1.3 ± 0.6 episodes) and was lowest from morning to late afternoon (8 AM to 4 PM: total 3 episodes, mean 0.2 ± 0.4 episodes). The frequency from late afternoon to midnight was higher than that from morning to late afternoon (4 PM to midnight: total 7 episodes, mean 0.4 ± 0.7 episodes).

In patients with VA, flow-mediated vasodilation was lowest in the early morning and was highest in the afternoon. Flow-mediated vasodilation in the evening tended to be lower than that in the afternoon (8 PM: 7.8 ± 2.1% [$p < 0.01$ vs. 6 AM value]; 6 AM: 5.4 ± 2.3%; 2 PM: 8.8 ± 1.9% [$p < 0.01$ vs. 6 AM value]). The control subjects showed a similar pattern, but the difference was not significant (8 PM: 9.5 ± 2.8%; 6 AM: 9.0 ± 2.2%; 2 PM: 9.9 ± 1.9%). Between

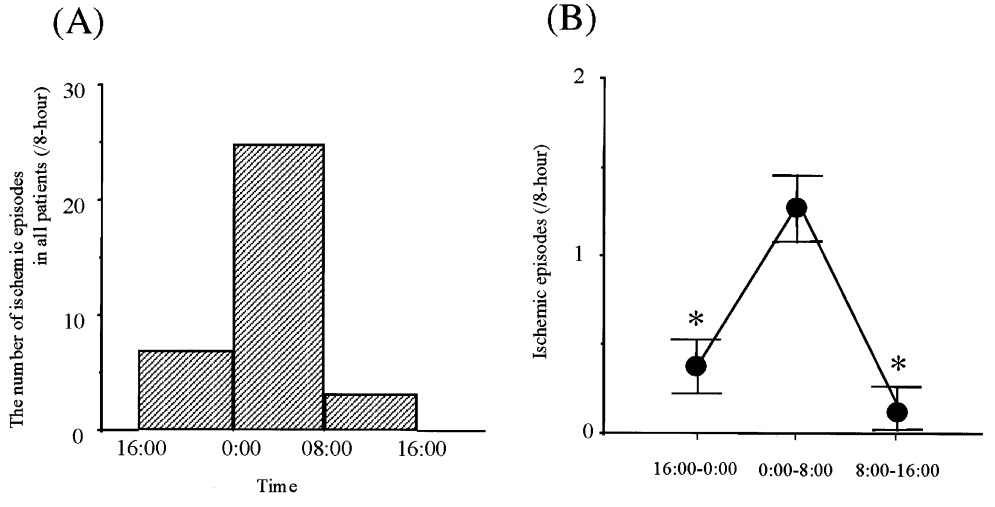

Figure 1. Diurnal variation of the total myocardial ischemic episodes in all patients with variant angina **(A)** and that of the mean myocardial ischemic episodes in each patient **(B)**. The frequency of ischemic episodes was highest from midnight to morning (00:00 to 08:00) and was lowest from morning to late afternoon (08:00 to 16:00). The frequency from late afternoon to midnight was higher than that from morning to late afternoon (16:00 to 00:00). *$p < 0.01$ vs. from midnight to morning (00:00 to 08:00). See text for details. 00:00 = midnight; 08:00 = 8 AM; 16:00 = 4 PM.

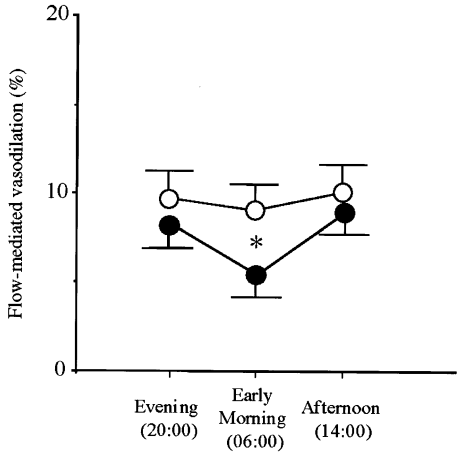

Figure 2. Fluctuation of flow-mediated, endothelium-dependent vasodilation in 20 patients with variant angina **(solid circles)** and 20 control subjects **(open circles)**. See text for details. *$p < 0.01$ by analysis of variance. 06:00 = 6 AM; 14:00 = 2 PM; 20:00 = 8 PM.

the two groups, flow-mediated vasodilation was comparable in the evening (7.8 ± 2.1% vs. 9.5 ± 2.8%; p = NS) and in the afternoon (8.8 ± 1.9% vs. 9.9 ± 1.9%; p = NS), but was less in the patients with VA in the early morning (5.4 ± 2.3% vs. 9.0 ± 2.2%; $p < 0.01$). The fluctuation in flow-mediated vasodilation was significantly different between the two groups ($p < 0.01$ by ANOVA), as shown in Figure 2. There was a negative correlation between flow-mediated vasodilation and the frequency of myocardial ischemic episodes (Fig. 3). Nitroglycerin-induced vasodilation (endothelium-independent vasodilation) remained unchanged throughout the three observations and was comparable between the two groups, as shown in Table 2.

DISCUSSION

In the present study, the frequency of ischemic episodes displayed diurnal variation in patients with VA, which is consistent with the results of our previous studies (2,7). Endothelial function of the brachial artery fluctuated in association with the frequency of ischemic episodes.

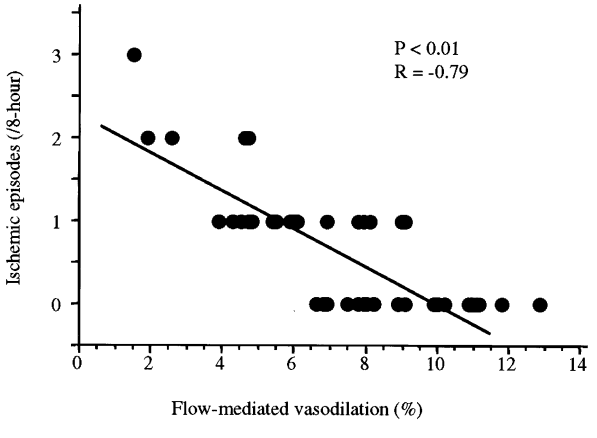

Figure 3. Relationship between flow-mediated, endothelium-dependent vasodilation and the frequency of ischemic episodes. See text for details.

Mechanism(s) of coronary spasm. The precise mechanism of coronary spasm is still unknown. There has been considerable debate regarding whether endothelial function is impaired or preserved at the site of coronary spasm (2–4,7,17–19). However, endothelium-dependent vasodilation, which plays a crucial role in the regulation of vessel tone, is known to be induced in response to various stimuli, including ACh, serotonin, ergonovine, histamine, blood flow and shear stress (20–22). In patients with coronary spasm, coronary dilation caused by these endothelium-dependent vasodilators is reported to be impaired (3–7,23). In addition, antioxidants, such as vitamin C and glutathione, attenuate the constrictor response to ACh (24,25). The plasma levels of vitamin E, a natural antioxidant, are decreased (26), and its oral administration reduces the myocardial ischemic episodes due to coronary spasm and improves the endothelium-dependent vasodilation of brachial arteries in patients with coronary spasm (12). In the present study, intracoronary injection of ACh caused coronary spasm in all patients with VA. Thus, patients with coronary spasm may have a disturbance in endothelial function of the coronary arteries, as well as a hypercontractile response of vascular smooth muscle (7).

We have further reported that the coronary arteries of patients with VA exhibit a circadian variation in tone (8), similar to the phenomenon observed in the present study. As the endothelial function of the brachial artery and that of the coronary artery are closely related (12), the fluctuation of endothelium-dependent dilation in the brachial artery most likely takes place in the coronary arteries as well.

Mechanism(s) of diurnal variation in flow-mediated vasodilation. The precise mechanism of this fluctuation remains undetermined in the present study. However, it is known that the day/night pattern in the intensity of physical activity causes diurnal variation of hemodynamics (27). In the evening and afternoon, peripheral blood flow to the skeletal muscle is increased through local regulation in proportion to its need for oxygen and nutrients (28). Likewise, in the present study, the baseline values of brachial artery blood flow were greater in the evening and afternoon than in the early morning in both groups. Endothelial nitric oxide synthase is known to be upregulated by increased blood flow or shear stress (29–31). Therefore, it is possible that the variation in baseline blood flow may upregulate nitric oxide synthase and contribute to the fluctuation of the flow-mediated, endothelium-dependent vasodilation in patients with VA. Diurnal fluctuation of endothelial function may play an important role in the occurrence of ischemic episodes in patients with VA.

In contrast, the endothelium-dependent vasodilation of the brachial artery in the control subjects also showed a similar pattern, but the difference was not significant in the present study. This may be supported by the finding that coronary segments with dysfunctional endothelium displayed morning exaggeration in their constrictor response to

ACh, whereas segments with normally functioning endothelium showed no such variation (32).

The present study demonstrated for the first time, to the best of our knowledge, that there is diurnal fluctuation in the endothelial function of patients with VA, which is associated with the frequency of ischemic attacks.

Reprint requests and correspondence: Dr. Hiroaki Kawano, Department of Cardiovascular Medicine, Kumamoto University School of Medicine, 1-1-1 Honjo, Kumamoto City 860-8556, Japan. E-mail: koumei@gpo.kumamoto-u.ac.jp.

REFERENCES

1. Hillis LD, Braunwald E. Coronary artery spasm. N Engl J Med 1978;299:695–702.
2. Yasue H, Omote S, Takizawa A, Nagao M. Coronary arterial spasm in ischemic heart disease and its pathogenesis. Circ Res 1983;52 Suppl I:I147–52.
3. Yasue H, Horio Y, Nakamura N, et al. Induction of coronary spasm by acetylcholine in patients with variant angina: possible role of the parasympathetic nervous system in the pathogenesis of coronary artery spasm. Circulation 1986;74:955–63.
4. Okumura K, Yasue H, Matsuyama K, et al. Effect of H1 receptor stimulation on coronary artery diameter in patients with variant angina: comparison with effect of acetylcholine. J Am Coll Cardiol 1991;17:338–45.
5. Kaski JC, Crea F, Meran D, et al. Local coronary supersensitivity to diverse vasoconstrictive stimuli in patients with variant angina. Circulation 1986;74:1255–65.
6. Mcfadden EP, Clarke JG, Davies GJ, Kaski JC, Haider AW, Maseri A. Effect of intracoronary serotonin on coronary vessels in patients with stable angina and patients with variant angina. N Engl J Med 1991;324:648–54.
7. Yasue H, Kugiyama K. Coronary spasm: clinical features and pathogenesis. Intern Med 1997;36:760–5.
8. Yasue H, Omote S, Takizawa A, Nagao M, Miwa K, Tanaka S. Circadian variation of exercise capacity in patients with Prinzmetal's variant angina: role of exercise-induced coronary artery spasm. Circulation 1979;59:938–48.
9. Okumura K, Yasue H, Matsuyama K, et al. Diffuse disorder of coronary artery vasomotility in patients with coronary spastic angina: hyperreactivity to the constrictor effects of acetylcholine and the dilator effects of nitroglycerin. J Am Coll Cardiol 1996;27:45–52.
10. Joannides R, Haefeli WE, Linder L, et al. Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. Circulation 1995;91:1314–9.
11. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 1991;43:109–42.
12. Motoyama T, Kawano H, Kugiyama K, et al. Vitamin E administration improves impairment of endothelium-dependent vasodilation in patients with coronary spastic angina. J Am Coll Cardiol 1998;32:1672–9.
13. Anderson TJ, Uehata A, Gerhard MD, et al. Close relation of endothelial function in human coronary and peripheral circulations. J Am Coll Cardiol 1995;26:1235–41.
14. Kawano H, Motoyama T, Kugiyama K, et al. Gender difference in improvement of endothelium-dependent vasodilation after estrogen supplementation. J Am Coll Cardiol 1997;30:914–9.
15. Corretti MC, Plotnick GD, Vogel RA. Technical aspects of evaluating brachial artery endothelium-dependent vasodilatation using high frequency ultrasound. Am J Physiol 1995;268:H1397–404.
16. Noma A, Okabe H, Netsu-Nakayama K, Ueno Y, Shinohara H. Improved method for simultaneous determination of cholesterol in high- and low-density lipoproteins. Clin Chem 1979;25:1480–1.
17. Egashira K, Inou T, Yamada A, Hirooka Y, Takeshita A. Preserved endothelium-dependent vasodilation at the vasospastic site in patients with variant angina. J Clin Invest 1992;89:1047–52.
18. Kuga T, Egashira K, Mohri M, et al. Bradykinin-induced vasodilation is impaired at the atherosclerotic site but is preserved at the spastic site of human coronary arteries in vivo. Circulation 1995;92:183–9.
19. Fukai T, Egashira K, Hata H, et al. Serotonin-induced coronary spasm in a swine model: a minor role of defective endothelium derived relaxing factor. Circulation 1993;88:1922–30.
20. Rubanyi GM, Romero JC, Vanhoutte PM. Flow-induced release of endothelium-derived relaxing factor. Am J Physiol 1986;250:H1145–9.
21. Furchgott RF, Zawadski JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980;288:373–6.
22. Pohl U, Holtz J, Busse R, Bassenge E. Crucial role of endothelium in the vasodilator response to increased flow in vivo. Hypertension 1986;8:37–44.
23. Kugiyama K, Ohgushi M, Motoyama T, et al. Nitric oxide-mediated flow-dependent dilation is impaired in coronary arteries in patients with coronary spastic angina. J Am Coll Cardiol 1997;30:920–6.
24. Kugiyama K, Motoyama T, Hirashima O, et al. Vitamin C attenuates abnormal vasomotor reactivity in spasm coronary arteries in patients with coronary spastic angina. J Am Coll Cardiol 1998;32:103–9.
25. Kugiyama K, Miyao Y, Sakamoto T, et al. Glutathione attenuates coronary constriction to acetylcholine in patients with coronary spastic angina. Am J Physiol Heart Circ Physiol 2001;280:H264–71.
26. Miwa K, Miyagi Y, Igawa A, Nakagawa K, Inoue H. Vitamin E deficiency in variant angina. Circulation 1996;95:14–8.
27. Veerman DP, Imholz BPM, Wieling W, Wesseling KH, Montfrans GA. Circadian profile of systemic hemodynamics. Hypertension 1995;26:55–9.
28. Jones RD, Berne RM. Intrinsic regulation of skeletal muscle blood flow. Circ Res 1964;14:126–38.
29. Sessa WC, Pritchard K, Seyedi N, Wang J, Hintze TH. Chronic exercise in dogs increases coronary vascular nitric oxide production and endothelial cell nitric oxide synthase gene expression. Circ Res 1994;74:349–53.
30. Noris M, Morigi M, Donadelli R, et al. Nitric oxide synthesis by cultured endothelial cells is modulated by flow conditions. Circ Res 1995;76:536–43.
31. Nishida K, Harrison DG, Navas JP, et al. Molecular cloning and characterization of the constitutive bovine aortic endothelial cell nitric oxide synthase. J Clin Invest 1992;90:2092–6.
32. El-Tamimi H, Mansour M, Pepine CJ, Wargovich TJ, Chen H. Circadian variation in coronary tone in patients with stable angina. Circulation 1995;92:3201–5.